

Familial Cryptic Translocation Resulting in Angelman Syndrome: Implications for Imprinting or Location of the Angelman Gene?

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Summary

Angelman syndrome (AS) is associated with a loss of maternal genetic information, which typically occurs as a result of a deletion at 15q11-q13 or paternal uniparental disomy of chromosome 15. We report a patient with AS as a result of an unbalanced cryptic translocation whose breakpoint, at 15q11.2, falls within this region. The proband was diagnosed clinically as having Angelman syndrome, but without a detectable cytogenetic deletion, by using high-resolution G-banding. FISH detected a deletion of *D15S11* (IR4-3R), with an intact *GABRB3* locus. Subsequent studies of the proband's mother and sister detected a cryptic reciprocal translocation between chromosomes 14 and 15 with the breakpoint being between *SNRPN* and *D15S10* (3-21). The proband was found to have inherited an unbalanced form, being monosomic from 15pter through *SNRPN* and trisomic for 14pter to 14q11.2. DNA methylation studies showed that the proband had a paternal-only DNA methylation pattern at *SNRPN*, *D15S63* (PW71), and *ZNF127*. The mother and unaffected sister, both having the balanced translocation, demonstrated normal DNA methylation patterns at all three loci. These data suggest that the gene for AS most likely lies proximal to *D15S10*, in contrast to the previously published position, although a less likely possibility is that the maternally inherited imprinting center acts in *trans* in the unaffected balanced translocation carrier sister.

Introduction

Angelman syndrome (AS) was first described by Dr. Harry Angelman (1965). Characteristic features include

severe mental retardation with lack of speech, ataxic gait and uncoordinated movements, seizures, hypopigmentation, microbrachycephaly, mandibular prognathism, and an inappropriate affect with frequent bouts of laughter. In an estimated 70%–75% of the cases, there is a deletion of maternal chromosome 15q11-q13 (Knoll et al. 1989; D. J. Driscoll, R. N. Nicholls, R. Z. Zori, and C. A. Williams, unpublished data). In individuals with Prader-Willi syndrome (PWS), a clinically distinct genetic condition, there is a loss of the paternally inherited chromosome 15q11-q13 (Butler et al. 1983; Knoll et al. 1989). The deletions found in individuals with these two syndromes overlap; however, it is felt that the genes involved in the pathogenesis of each syndrome are distinct (Saitoh et al. 1992; Buxton et al. 1994; Sutcliffe et al. 1994). The crucial region for PWS lies centromeric to that of AS (Saitoh et al. 1992) and is believed to include the *SNRPN* gene (Glenn et al. 1993b; Sutcliffe et al. 1994). Buxton et al. (1994) describe a single patient with typical AS who was deleted for the maternal *D5S113* locus, but not for the flanking loci *D15S10* and *GABRB3*, and propose that the candidate gene for AS is limited to an area of 200 kb around *D15S113*. Since the publication of this data, it has been determined that this patient does not have a microdeletion of the *D15S113* locus but that one of the alleles is a null allele, which yielded confusing results (J. L. Buxton, personal communication).

The parent-of-origin distinction between AS and PWS is a result of genomic imprinting of genes within 15q11-q13 (Driscoll 1994). DNA methylation imprints have been described at three loci within the 15q11-q13 region; *ZNF127* (Driscoll et al. 1992), *D15S63* (PW71) (Dittrich et al. 1992) and *SNRPN* (Glenn et al. 1993b, 1996; Sutcliffe et al. 1994). These loci are differentially methylated and distinguish the maternally and paternally inherited alleles. *SNRPN* is functionally imprinted, with only the paternal copy transcriptionally active (Glenn et al. 1993b), and therefore may contribute to the pathogenesis of the PWS phenotype.

About 20%–30% of classical AS patients have neither a 15q11-q13 deletion or paternal UPD (Clayton-

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Smith et al. 1992; D. J. Driscoll, R. N. Nicholls, R. Z. Zori, and C. A. Williams, unpublished data). A subset of this group has paternal-only DNA methylation patterns at loci within the 15q11-q13 region (Glenn et al. 1993a; Reis et al. 1994; Buiting et al. 1995). In these patients, the phenotype appears to be a result of deletions in the imprinting center that affect the expression of the putative AS gene. We present a child with AS associated with an unbalanced cryptic translocation in which the deletion involves *SNRPN* and all loci proximal to that but does not extend to the region believed to contain the critical gene(s) involved in the pathogenesis of AS (Buxton et al. 1994). The implications of the deletion involved in this family are discussed with regard to the diagnosis of cryptic translocations and genomic imprinting, as well as proposing an alternate region for the AS gene.

Subjects, Material, and Methods

Subjects

The proband was a 10-year-old girl who was diagnosed with AS at the age of 7 10/12 years. She had the following features: extreme short stature (at 10 years of age, her height age was 4 3/12 years), severe mental retardation, lack of speech, seizures, microbrachycephaly (frontal occipital circumference $\ll 2$ SD below the mean), prominent mandible, an inappropriately happy affect with frequent episodes of laughter, and a wide-based, ataxic gait (fig. 1). Her past medical history included irritability as a newborn, left-sided diaphragmatic paralysis, and severe gastroesophageal reflux with aspiration, resulting in a fundoplication, an adenoidectomy for chronic mouth breathing and congestion, and multiple sets of myringotomy tubes. She had multiple abnormal electroencephalograph (EEG) recordings felt to be consistent with a static encephalopathy. Routine cytogenetic and high-resolution banding studies were done and revealed no abnormalities. At 10 years the proband was hospitalized for seizures, and her EEG revealed generalized and asymmetric left frontal sharp wave activity with occasional spikes, consistent with the pattern observed in older children with AS (Boyd et al. 1988; Clayton-Smith et al. 1993). At 10 years of age there were no clinical features felt to be inconsistent with the diagnosis of AS. During that hospitalization blood was obtained for analysis by FISH using DNA probes from 15q11-q13.

Family studies were done on blood from the proband's only sibling, her mother, maternal uncle, maternal aunt, maternal great aunt, and maternal great uncle. The proband's father was not available and the maternal grandparents are deceased. The phenotypes of all relatives studied were unremarkable, except for the maternal great aunt who had mild-to-moderate mental retarda-

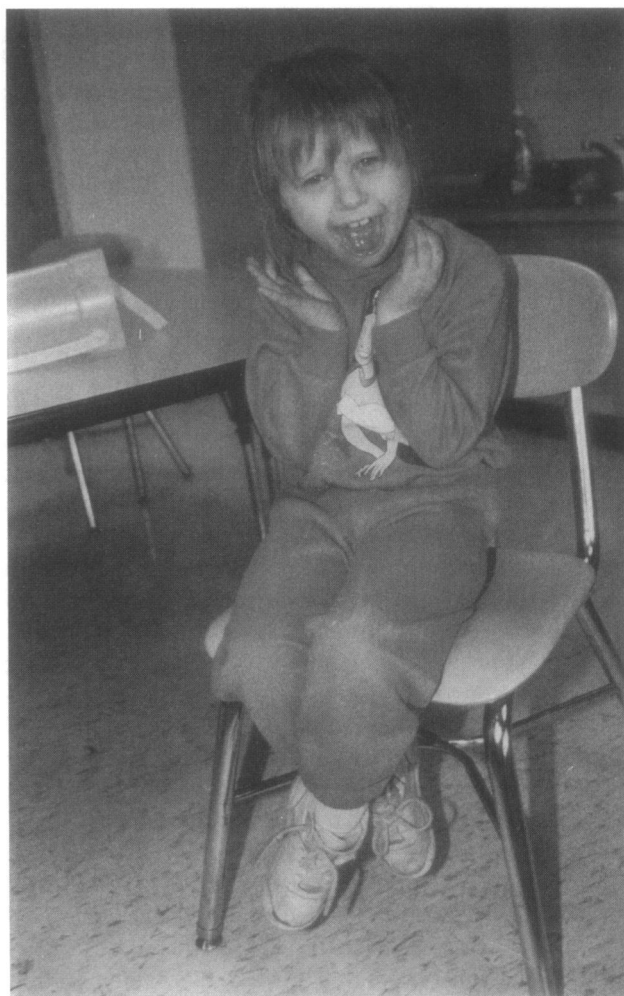


Figure 1 Proband at age 10 years. Note the mandibular prognathism and happy affect.

tion of unknown etiology without apparent dysmorphism.

Cytogenetic Studies

Peripheral blood was processed by standard procedures to yield preparations for cytogenetic analysis. Chromosomes were G-banded using trypsin and Wright's stain.

FISH

All probes utilized in this study were obtained from Oncor. These included cosmid probes for Prader-Willi/Angelman syndrome regions A (*D15S11*) and B (*GABRB3*), chromosome 14/22 α -satellite, *SNRPN* and *D15S10* probes. Photographs were taken with Kodak Gold 400 color print film.

DNA Methylation

Parent-of-origin DNA methylation imprints were determined using the DN34 probe from the *ZNF127* gene

(Driscoll et al. 1992); the *D15S63* (PW71) probe (Dittrich et al. 1992); and a probe from the 5' end of *SNRPN* (Glenn et al. 1996). Methylation analysis was performed as described elsewhere (Driscoll et al. 1992). Ten micrograms of DNA were first digested with a non-methyl-sensitive enzyme (*EcoRI* for *ZNF127*, *HindIII* for *D15S63*, and *XbaI* for 5' *SNRPN*) and then digested with a methyl-sensitive enzyme (*HpaII* for *ZNF127* and *D15S63*, and *NotI* for 5' *SNRPN*). DNA was run on agarose gels, Southern blotted, and hybridized with the appropriate cloned DNA sequence. Autoradiographs were exposed at -70°C by using intensifying screens and then developed in a standard x-ray processor.

Microsatellite Analysis

Microsatellite analysis was performed according to a variation of the technique described by Mutirangura et al. (1993a, 1993b) using the primers described by these authors at the following loci: *D15S11* (IR4-3R), *D15S113* (LS6-1), and *GABRB3*.

Results

Conventional cytogenetic studies of the proband in 1990 revealed an apparently normal female karyotype. When the Prader-Willi/Angelman syndrome cosmid probes became available, the proband was restudied using FISH. Using the *D15S11* (IR4-3R) probe, two chromosomes demonstrated hybridization with the control probe that maps to the distal region of chromosome 15, but only one chromosome had a signal from the region of *D15S11*, indicating a deletion of this region on one of the chromosome 15 homologues. FISH using the *GABRB3* probe revealed hybridization to two chromosomes.

The mother of the proband was subsequently studied with conventional cytogenetics and FISH using the *D15S11* and *GABRB3* probes. An apparently normal female karyotype was seen using conventional G-banded chromosomes. FISH, however, revealed that one signal from *D15S11* was on a different D group acrocentric chromosome than the signal from the chromosome 15 control probe. Dual color FISH was performed with the chromosome 15 probes (*D15S11* and *PML* control) and 14/22 α -satellite probes, defining a cryptic, apparently balanced translocation between chromosomes 14 and 15 (fig. 2a).

The proband was also studied utilizing dual color FISH and was found to have inherited a derivative chromosome 14 containing the short arm, centromere, and a small portion of the long arm of chromosome 14 to q11.2 as well as most of the long arm of chromosome 15 (15q11.2→qter) (fig. 2b). When the proband's sister was studied, conventional cytogenetics failed to reveal an abnormality, but FISH showed that she and her

mother carried an apparently balanced translocation between chromosomes 14 and 15.

Subsequent studies were undertaken of the proband and her mother and sister with the *SNRPN* and *D15S10* probes. The *D15S10* probe (fig. 3a, b) revealed two chromosomes each with a *PML* signal distal to the *D15S10* signal in the proband as well as her mother and sister. However, the *PML* signal appeared on a different D group acrocentric chromosome from the *SNRPN* probe in the mother and sister (fig. 3c). In the proband, there were two *PML* signals and only a single signal from *SNRPN* (fig. 3d). These studies indicate that the breakpoint occurs between *D15S10* and *SNRPN* (fig. 4).

The balanced translocation found in the mother and sister is represented by the karyotype 46,XX,t(14-15)(q11.2-q11.2). The karyotype of the proband is 46,XX,-15,+der(14) t(14-15)(q11.2-q11.2)mat.

DNA methylation analyses on the proband revealed a paternal-only methylation pattern for the *ZNF127* and *SNRPN* genes (fig. 5), as well as the *D15S63* locus. The sister and mother showed a normal biparental DNA methylation pattern at all three loci. To confirm the FISH results, microsatellite analyses were performed at the *D15S11*, *D15S113*, and *GABRB3* loci. The results in figure 6 demonstrate that the proband has a maternal deletion at *D15S11* (IR4-3R) but has two alleles at *D15S113* (LS6-1) as well as at *GABRB3*.

Discussion

The family presented here, as well as several other reported cases (Hultén et al. 1991; Tepperberg et al. 1993), demonstrates that in rare cases of AS and PWS, the deletions are detectable only by FISH and may be a result of a cryptic translocation between chromosome 15 and another acrocentric chromosome in one of the parents. The discovery of an unbalanced translocation in this patient was made only when FISH studies on the maternal chromosomes uncovered an unusual pattern. This finding suggests that an α -satellite probe for chromosome 15 should be used in AS and PWS cases where a deletion has been found in conjunction with the 15q11-q13 and distal control (*PML*) probes to uncover cryptic translocations, particularly in cases in which the parental chromosomes reveal an unusual pattern. In the absence of this testing, a case could be mislabeled as sporadic, when a parent is a carrier of a balanced cryptic translocation. Pairing at meiosis for carriers is most likely to be as two independent bivalents (i.e., the normal 14 with the derivative 15, and the normal 15 with the derivative 14) rather than as a quadrivalent. Thus the theoretical risk of offspring with an unbalanced genome from such parents is as high as 50% (25% for a deletion in 15q11-q13).

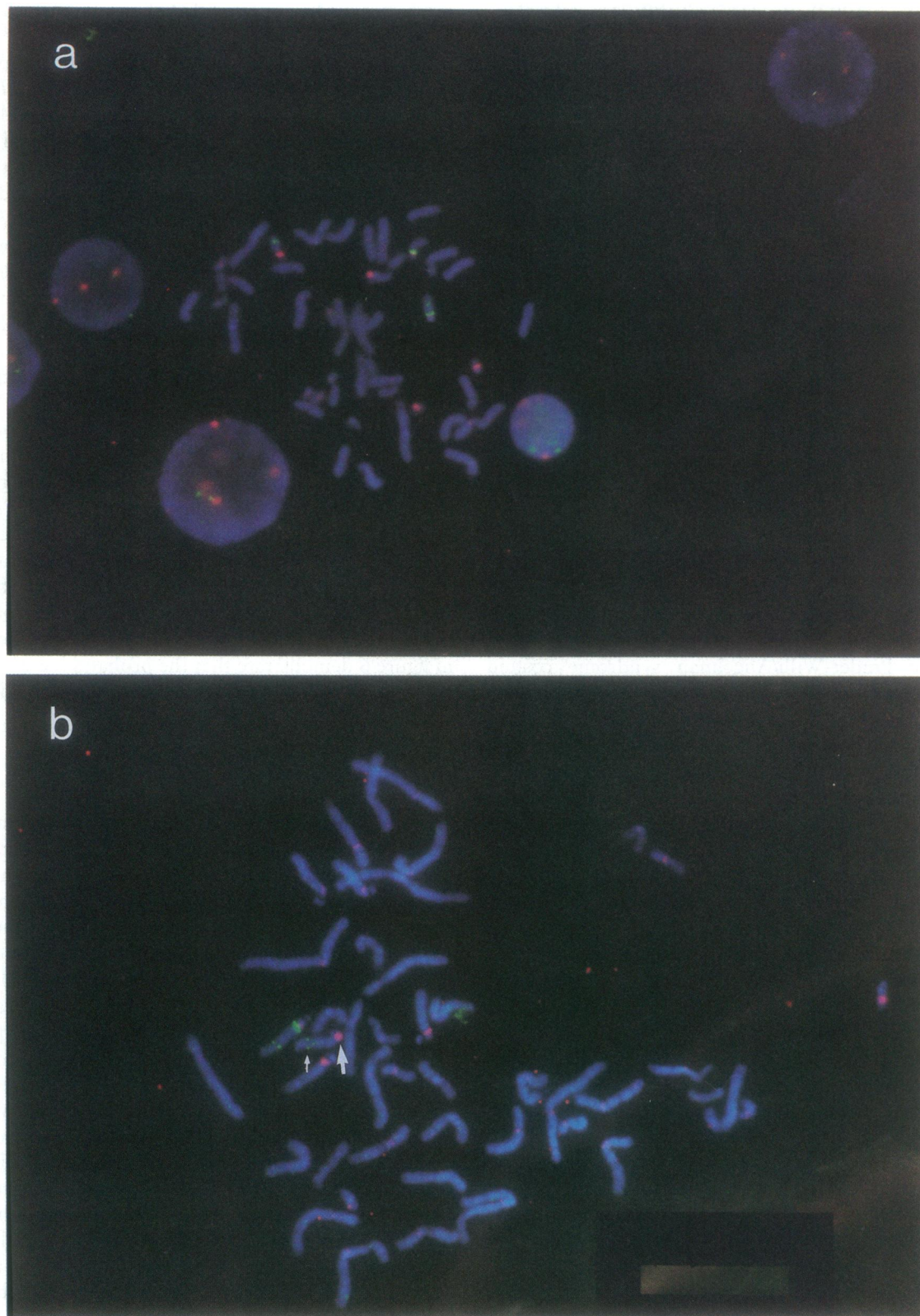


Figure 2 Dual color FISH with a 14/22 α -satellite probe (red) and the chromosome 15 probes, *D15S11* and *PML* (both green). *a*, Proband's mother's chromosomes. The chromosome 15 *PML* locus has been translocated to the derivative chromosome 14. *b*, Proband's chromosomes. The derivative chromosome 14 has the chromosome 15 *PML* marker (*small arrow*) and the 14 centromere (*large arrow*).

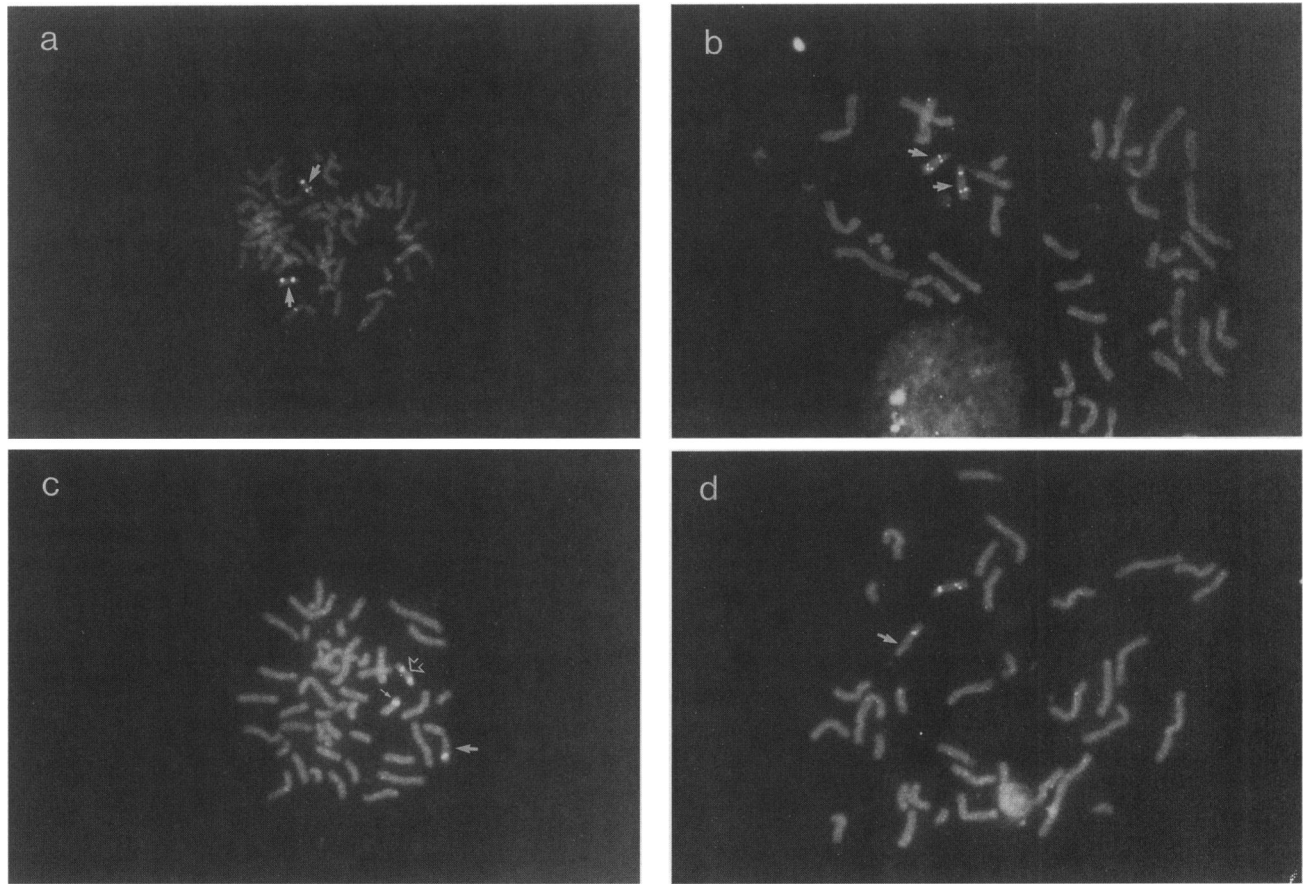


Figure 3 FISH using *D15S10* and *SNRPN* probes. *a*, Proband's mother's chromosomes, using *D15S10* and control *PML* probes. Note that the *D15S10* and the *PML* probes hybridize to two D group chromosomes (arrows). *b*, Proband's chromosomes, using *D15S10*. Note that the *D15S10* and the *PML* signals are on the same chromosomes (arrows). *c*, Proband's mother's chromosomes, using the *SNRPN* probe. Note that one copy of the *SNRPN* locus (small arrow) is on a separate acrocentric chromosome from the *PML* control probe (large arrow). The intact chromosome 15 is indicated by the open arrow. *d*, Proband's chromosomes, using the *SNRPN* probe. Note that the *PML* probe is present on two acrocentric chromosomes, but the *SNRPN* signal is only present on one chromosome (arrow).

As shown in figure 4, the breakpoint of the translocation found in this family lies between *SNRPN* and *D15S10*(3-21). The proband is trisomic for 14 pter to 14q11.2 and monosomic for 15pter to 15q1. Therefore, the proband has only the paternal copy of the *ZNF127* (*D15S9*), *D15S11* (IR4-3R), *D15S13* (189-1), *D15S63* (PW71), and *SNRPN* loci. Parent-of-origin-specific DNA methylation has been demonstrated at *SNRPN* as well as at the *ZNF127* and *D15S63* loci (Dittrich et al. 1992; Driscoll et al. 1992; Glenn et al. 1993a, 1993b, 1996; Sutcliffe et al. 1994). The proband described here shows only a paternal methylation pattern at all of these loci.

Only the paternal allele of *SNRPN* is expressed (Glenn et al. 1993b; Nakao et al. 1994; Reed and Leff 1994) and is, therefore, a candidate gene for at least part of the pathogenesis of PWS. Sutcliffe et al. (1994) describe a patient with PWS in whom the deletion involves the most 5' exon of *SNRPN* and extends proxi-

mally on the paternally inherited chromosome 15. This deletion, in which the most 5' exon and the putative promoter are deleted, not only disrupts the imprinted expression of *SNRPN*, but also the expression of two other paternally expressed transcripts, *PAR1* and *PAR5*, even though they are intact and biparental in origin (Sutcliffe et al. 1994).

There are at least four mechanisms by which the AS phenotype can occur: deletion of the maternal 15q11-q13; paternal uniparental disomy for chromosome 15; a deletion or mutation in the maternal imprinting center; and presumably a mutation in the maternal gene(s) whose absence of expression causes AS (Driscoll 1994). Various workers (Glenn et al. 1993a; Reis et al. 1994; Sutcliffe et al. 1994) have found imprinting mutations in both AS and PWS. Recently, Buiting et al. (1995) have shown that these AS and PWS patients have small (40–50 kb) deletions proximal to *SNRPN*, thus localizing to this region an imprinting center for both the ma-

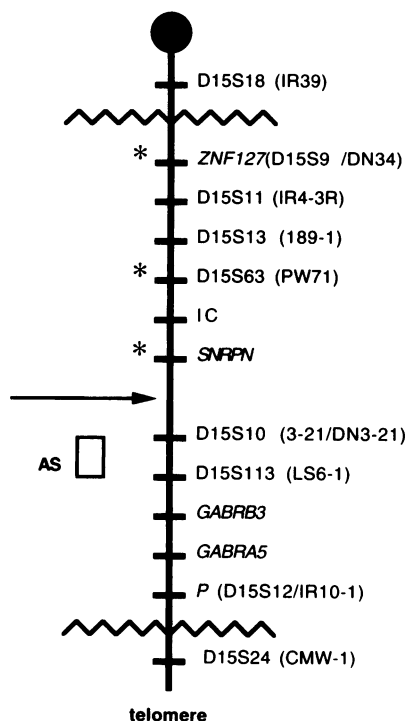


Figure 4 The order of the DNA probes from the chromosome 15q11-13 region (adapted from Mutirangura et al. 1993b). The smallest AS deletion reported (Buxton et al. 1994) is indicated by the box. The zigzag lines proximal to *ZNF127* and distal to *P* represent the deletion region for the majority of AS and PWS patients. The asterisks (*) denote loci with parent-of-origin DNA methylation imprints. The arrow indicates the approximate position of the translocation breakpoint in this family.

ternally and paternally inherited chromosome 15. Imprinting center mutations are thought to block the resetting of the imprint in gametogenesis (Buiting et al. 1995). However, the stage of gametogenesis in which the imprint is reset and how it is maintained in somatic cells is currently unknown.

In addition to *SNRPN*, *PAR1*, and *PAR5*, two other genes in 15q11-q13, *ZNF127* (Jong et al. 1994) and *IPW* (Wevrick et al. 1994), have been shown to have paternal-only expression. However, no gene has currently been identified that is expressed only from the maternally inherited chromosome 15 and that would therefore be a candidate gene for AS. Since DNA methylation imprints are characteristic of imprinted genes (Razin and Cedar 1994), we would predict that the putative AS gene will also have a DNA methylation imprint. At least part of the candidate gene for AS is hypothesized to lie in the 200 kb surrounding the locus *D15S113* (LS61), on the basis of a single patient (Buxton et al. 1994), the results of which are now brought into question, as mentioned above (J. L. Buxton, personal communication). The patient described here has two alleles at this locus (fig. 6) but is deleted on the maternally

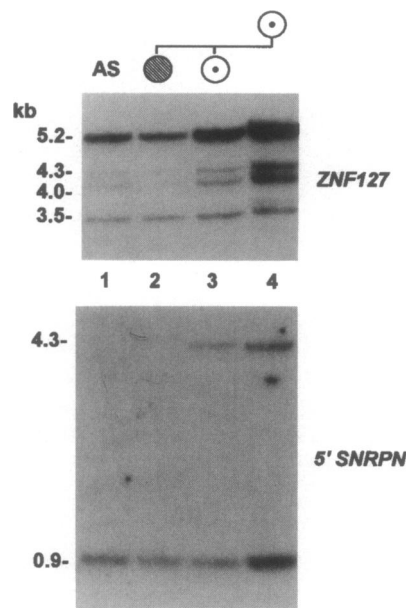


Figure 5 DNA methylation studies on the proband, her sister, and her mother. Note the paternal-only DNA methylation pattern at both *ZNF127* and 5' *SNRPN* for the proband (lane 2), while the mother and sister have a normal, biparental methylation pattern at these loci.

inherited chromosome 15 proximal to *D15S10* and including the *SNRPN* gene. Three loci in this region, *ZNF127*, *D15S63* (PW71), and *SNRPN*, were shown to have only the paternal methylation pattern present in the proband (fig. 5).

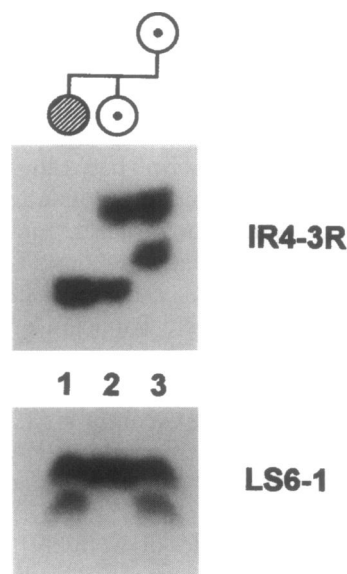


Figure 6 Microsatellite analysis using primers at the *D15S11* (IR4-3R) and *D15S113* (LS6-1) loci. The proband has two alleles at *D15S113* (LS6-1) and only one at *D15S11* (IR4-3R), indicating a maternal deletion including the latter locus.

There are at least two possible mechanisms to explain the Angelman syndrome in the proband and the normal phenotype in the sister with the reciprocal translocation. The first possibility involves the loss of the imprinting center (IC). If the mother's translocation is on her paternally inherited chromosome 15, and if resetting the imprint does not typically occur until after metaphase I in oogenesis, then her germ cells would not be able to reset the 15q11-q13 imprint in the absence of the chromosome 15 IC. Thus, the proband would inherit a paternal imprint for the AS gene rather than the maternal imprint that would be necessary for normal expression for this imprinted gene. The proband's sister, who is clinically normal, has a balanced reciprocal translocation that would separate the maternally inherited IC gene from the gene it presumably imprints, assuming the location of the AS gene is where Buxton et al. (1994) suggest. This would imply that imprinting can act in *trans* and occurs after metaphase I in oogenesis, at least for maternal 15q11-q13. Its mechanism of action would thus be different than X-inactivation, which acts in *cis* (Gartler et al. 1992).

A second possibility is that the AS gene is actually located between *SNRPN* and *D15S10*. Thus, the proband is affected because she is deleted for the maternally inherited part of chromosome 15 proximal to *D15S10* that would include both the IC and the AS gene. The normal sister would have the maternally inherited IC and AS gene on the same chromosome. This scenario is thus compatible with the imprinting center working in *cis*.

There has been no evidence presented in the literature that an imprinting center can work in *trans*. In addition, there have been no reports by other authors that support the finding of Buxton et al. that the Angelman gene lies within the 200 kb surrounding the *D15S113* locus, and subsequent studies indicate that Buxton's patient does not have the microdeletion previously reported. Therefore, we believe that the second of the possibilities presented above is the most likely. That is, the locus for the gene for AS is proximal to *D15S10*, and the AS phenotype in the proband occurs as a result of a deletion of the maternal AS gene rather than a disturbance of the imprinting mechanism. The normal phenotype of the proband's sister would then be expected, since she would have her maternal IC and AS gene on the same chromosome. It will be crucial to verify the location of the gene whose absence on the maternally inherited chromosome 15 results in AS. We believe that the individuals described here will be helpful in this regard.

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